MICROSCOPY.[1]

Preservation of some Marine Animals.—In 1891 there appeared a paper by T. Tullberg, *Ueber Konserviering v. Evertebraten in ausgedehnten Zustand*, in which a novel use of magnesium sulphate, or Epsom salts, was described. Tullberg was guided in his researches by the *a priori* consideration, that, as sea-water contains several salts in definite proportions, it is probable that marine animals would not contract if the proportion of one of the salts was increased, for the animal is already accustomed to these substances; and, on the other hand, it might have a toxic effect. Experimenting with Actinia, he finds that chloride of sodium has no effect, but with sulphate or chloride of magnesium the Actinian expands its tentacles, and after a certain time does not contract at all when its tentacles are pinched. He lets the Actinian expand in a vessel of sea-water, the quantity being determined so that the percentage of the salt added may be known. He then adds to the vessel a thirty-three per cent solution of magnesium chloride or sulphate until the water contains 1% of the salt. The additon is made slowly but is effected within half an hour, at the end of which time the Actinian is found to be anæsthetized. As a matter of fact only the exterior of the animal loses its sensibility.

It is then necessary to kill the animal which may be done by inundating it with some killing fluid, but in this case partial contraction may take place rendering it unfit for museum purposes. A better method is to kill it by slowly adding a 0·1% solution of chromic acid until the water contains from ·03% to ·05% of the acid. The results of this method are very satisfactory save that there is a decrease in the volume of the animal. Sections of the tentacles showed that the cells were not attacked by the substances employed.

This method was applied successfully to various fresh-water and salt-water invertebrates including various Actinians Holothurian, Turbellarians, Nemertines, Chætopods, Gasteropods, Ciona, etc. etc.

Last summer, through the kindness of Commissioner MacDonald, I had the opportunity of spending a few weeks at the U. S. Fish Commission laboratory at Wood's Hole, Mass., and obtained some interesting results with Epsom salts in the preservation of many of the marine invertebrates of that vicinity. The method of application requires modification in individual cases but a few experiments will usually en-

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able one to obtain the desired results and in a much simpler manner than that described by Tullberg. Complete stupification of the organism must be produced, so that when it is removed to a killing fluid, no contraction will take place. Care should be exercised, however, not to carry on the process too slowly as maceration may ensue.

CŒLENTERATES.—The most beautiful results were obtained with sea-anemones which ordinarily are so difficult to preserve in a well expanded condition. These were allowed to expand in a dish with as little water as possible. Then crystals of magnesium sulphate were placed in the bottom of the dish and allowed to dissolve slowly until a saturated solution was obtained. The process of dissolving may be hastened if necessary by stirring up the water gently from time to time with a pipette. Several hours were required to completely stupify large specimens. When narcotization was complete, a few crystals placed in the mouth of the sea-anemone had no effect but if the process had not gone far enough the lips of the animal would slowly spread open and then would follow sometimes a violent contraction of the whole animal. This method was tried upon *Metridium marginatum*, *Sagartia leucolena* and *Halocampa producta* with excellent results, the tentacles remaining perfectly expanded after the animals had been transferred to Perenyi's fluid, picro-sulphuric acid or formalin. The same method applied to *Astrangea*, *Scyphistoma*, and various hydroids did not give as good results as those obtained with the sea-anemones. The polyps were not equally affected so that only portions of the colonies were perfectly expanded. A large Physalia treated in this way was preserved in 4% formalin with all the tentacles and polyps fully extended.

ECHINODERMS.—Star-fishes and sea-urchins were killed with the ambulacral feet and pedicellaria well extended, by placing them upon the aboral surface for a short time in a saturated solution of Epsom salts and then transferring them to 4% formalin. The epidermis of the star-fishes, however, was rendered soft and was subsequently easily rubbed off, but this was probably due to the formalin.

Specimens of *Synapta* were readily preserved without any constriction by very slowly and intermittently adding to the water, in which they had been allowed to expand, a saturated solution of $MgSO_4$.

VERMES.—Most annelids when placed in a saturated solution of Epsom salts, in a very short time became perfectly limp and were easily extended upon a glass plate and treated with a fixing reagent. *Balanoglossus*, when taken soon after being collected, was preserved in this manner in nearly a perfect state. It was necessary, however, to keep it in position between the edges of two glass slides when the fix-

ing fluid was applied. Good results were obtained with *Cirratulus*, *Amphitrite*, *Nereis*, *Rhyncobolus*, *Clymenella* and *Phascolosoma*. *Phascolosoma* in most cases was killed with tentacles protruded. *Nemertean* worms, when transferred to a killing fluid before being completely narcotized, sometimes protruded their probosces.

ASCIDIANS.—*Molgula* and *Cynthia* were readily killed with siphons open after anæsthetization with magnesium sulphate. In this case it is best to add the saturated solution of sulphate intermittently with a pipette.

CTENOPHORES.—After considerable experimentation a method for preserving these delicate creatures in a nearly life-like appearance was devised. Formalin alone in solutions of varying strength had been tried without success. It was found necessary to treat the animals with some hardening reagent before placing them in the formalin and the following method seems to be the most successful. To a solution of equal parts of 2% formalin and Perenyi's fluid was added enough common salt (NaCl) to increase the density of the mixture to that of sea-water, i. e., until a Ctenophore placed in it barely floated. This adjustment of the density of the surrounding medium prevented the Ctenophores from collapsing of their own weight. After remaining for about half an hour in this fluid, they were transferred to 4% formalin, the density of which had been increased by the addition of either Epsom salts or common salt so that the Ctenophores again barely floated. Epsom salts is probably better than common salt for increasing the density of the fluid. Some specimens which were preserved in formalin+NaCl began to shrink after a few days, while some (Mnemiopsis) which have been preserved for nearly six months in formalin+$MgSO_4$ are still in excellent condition.

After the Ctenophores have been properly preserved, precaution must be taken in transporting them, for they are easily torn to pieces. If they are placed in bottles filled with fluid of the proper density and the cork so inserted as to leave no air bubbles, this danger is reduced to a minimum.—W. A. REDENBAUGH, Dartmouth Coll., Hanover, N. H.